Transglutaminase Autoantibodies in Dermatitis Herpetiformis and Celiac Sprue

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Dermatitis herpetiformis (DH) is an autoimmune blistering skin disorder that is associated with intestinal gluten sensitivity. Epidermal transglutaminase (TGe) and closely related tissue transglutaminase (tTG) are considered to be autoantigens in DH, because a majority of DH patients have IgA specific for TGe and for tTG. It is unknown where and how these autoantigen-specific IgAs are generated in DH. Results reported in this paper on nine DH patients on a gluten containing diet demonstrate that the levels of circulating anti-tTG IgA and anti-TGe IgA in DH are correlated with each other and that both appear to be correlated with the degree (extent) of enteropathy. An analysis of 15 untreated celiac sprue (CS) patients demonstrated that approximately 33% of CS patients had elevated levels of anti-TGe IgA. These results would indicate that intestinal damage is associated with the production of anti-tTG IgA and anti-TGe IgA in DH patients.

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INTRODUCTION

Dermatitis herpetiformis (DH) is an autoimmune, blistering, intensely pruritic papulovesicular rash typically located on the elbows, forearms, buttocks, knees, and scalp (Hall, 1992; Fry, 2002). It is often associated with an enteropathy characterized by villous atrophy and/or increased infiltration of intraepithelial lymphocytes (Fry, 1995; Cooney *et al.*, 1977). The enteropathy and the rash are caused by the ingestion of gluten, which is a group of storage proteins of wheat, barely, and rye. Another gluten sensitive disease, celiac sprue (CS), results from a potent inflammatory response to gluten within the small intestine (Reunala, 1998). Additionally, even though gluten is the exogenous antigen for both CS and DH, these two diseases have characteristics of autoimmune disorders. These characteristics include a tight association with major histocompatibility complex II molecules (HLA-DQ2 and HLA-DQ8) and the production of circulating autoantibodies (Spurkland *et al.*, 1997; Sollid, 2000).

In 1984, Chorelezki reported that autoantibodies directed against endomysial tissue were present in both DH and CS and could be used as a marker for both diseases (Chorzelski *et al.*, 1984). Subsequently, it was found that these endomysium-binding autoantibodies were directed specifically against tissue transglutaminase (tTG) (Dieterich *et al.*, 1997; Dieterich *et al.*, 1999; Porter *et al.*, 1999). The presence of circulating anti-tTG IgA is commonly used as a screening tool for celiac disease (James, 2005). Anti-tTG IgA antibodies are also diagnostic markers for enteropathy in DH patients (Kumar *et al.*, 2001). Thus, anti-tTG IgA is an autoantibody that is found in both diseases.

Interestingly, DH patients with villous atrophy have high levels of circulating anti-endomysial IgA (Volta *et al.*, 1992). Levels of circulating anti-tTG IgA and anti-endomysial IgA are correlated with the absence or presence of enteropathy in CS patients, suggesting that these antibodies are produced in the setting of mucosal injury (Kotze *et al.*, 2003; Rostami *et al.*, 2003). Yet another study demonstrated that IgA deposits and tTG colocalize in the jejunal samples of celiac patients and that this IgA was tTG specific based on binding studies of eluted jejunal IgA (Korponay-Szabo *et al.*, 2004).

In 2002, another autoantigen, epidermal transglutaminase (TGe), was identified for DH (Sardy *et al.*, 2002; Karpati, 2004). In the perilesional tissue of DH patients, the IgA deposits at the dermal/epidermal junction were found to colocalize with TGe in the papillary dermis and small vessels (Karpati, 2004; Preisz *et al.*, 2005). DH patients also had circulating TGe-specific IgA that fell into two groups. One antibody group bound to TGe exclusively, whereas the second antibody group was crossreactive and bound to both tTG and TGe. This second group was found in celiac patients as well and had a lower avidity for TGe than the first group. The level of circulating TGe-specific IgA was lower in DH patients on a gluten-free diet. This suggests that anti-TGe IgA is also dependent upon the continued intestinal exposure to gluten in DH patients, similar to anti-tTG IgA in CS patients.

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Abbreviations: CS, celiac sprue; DH, dermatitis herpetiformis; tTG, tissue transglutaminase; TGe, epidermal transglutaminase

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It is of interest then, as to whether the levels of anti-TGe IgA or anti-tTG IgA that are found in DH or CS patients are related to the degree or extent of mucosal injury, such as villous atrophy, as a result of intestinal exposure to gluten. To determine whether there is a correlation between the levels of circulating anti-TGe IgA and enteropathy/villous atrophy in DH and CS patients, we compared the titers of anti-TGe IgA and anti-tTG IgA with the presence and severity of villous atrophy.

RESULTS

Characterization of DH and CS patients

Nine DH patients that had not been on a gluten-free diet were evaluated for this study (Table 1). Duration of blistering disease varied from less than 0.5 years to 9 years (Table 1). Fifteen untreated celiac patients who had never developed a pruritic rash were evaluated (Table 2).

Correlation between the levels of anti-TGe IgA and anti-tTG IgA in DH and CS patients

The levels of anti-TGe IgA and anti-tTG IgA were highly correlated in DH patients, with a Pearson's coefficient, $r=0.74$. (Figure 1a). There was no significant correlation between the two autoantibodies in the untreated celiac patients ($r=0.286$, Figure 1b).

Relationship between enteropathy and serology

Marsh Scores were determined from the histopathological analysis of the duodenal biopsies of six of the nine DH patients and all of the 15 celiac patients. The scores varied from 0 to 3c (Tables 1 and 2). All biopsies were evaluated using the Marsh system of scoring (Oberhuber, 2000).

With the DH patients, there was a significant correlation between the levels of anti-TGe IgA and the degree of enteropathy ($r=0.82$) (Figure 2a). A significant correlation was also found between the marsh scores and levels of anti-tTG IgA ($r=0.7$) (Figure 2b). There was no correlation between the levels of anti-TGe IgA and the level of villous atrophy in the celiac patients (Figure 2a). Anti-tTG IgA also does not correlate with the degree of villous atrophy in CS patients (Figure 2b). However, all 15 celiac patients had elevated levels of anti-tTG IgA, thus supporting previous reports that elevated levels of anti-tTG IgA do correlate with the presence of enteropathy.

DISCUSSION

Several lines of evidence support TGe as a target for IgA autoantibodies in DH. Sardy *et al.* (2002) demonstrated that the IgA deposits in the perilesional skin of DH patients colocalizes with TGe, that DH and CS patients had circulating anti-TGe IgA in their blood, and that DH and CS patients on a gluten-free diet had lower levels of anti-TGe IgA than those that were not. One possible explanation for this is that the enteropathic process leads to the production of circulating anti-TGe IgA in both CS and DH patients.

Our results suggest a correlation among the extent of enteropathy, anti-tTG IgA and anti-TGe IgA in DH patients. Albeit, greater numbers of DH patients that are on a gluten containing diet would be necessary for definite proof of this correlation. This result would suggest that the production of these antibodies occurs as a result of mucosal damage in the intestine of DH patients. This would certainly fit the accepted theory that all DH patients have some level of intestinal immunopathological response to gluten that manifests as a skin condition.

Another important conclusion to be made from these results is that some celiac patients produce anti-TGe IgA, which supports a previous finding (Sardy *et al.*, 2002). The production of this antibody in celiac patients, however, did not appear to be correlated with severity of villous atrophy in their intestine. Similarly, the production of anti-tTG IgA in celiac patients did not correlate with the severity of enteropathy, which would also support a previous finding (Tursi *et al.*, 2003). One contributing factor to this lack of correlation between the production of anti-tTG and anti-TGe antibodies and the severity of enteropathy may be the fact that CS patients on a gluten-containing diet by definition have Marsh scores of 3 or greater, whereas all levels of severity can be present in DH patients on a gluten containing diet. Overall, however, these results would indicate that the

Table 1. Evaluated DH patients

DH patient no.	Age (years)	Sex	Disease duration (years)[1]	Marsh score	Villous atrophy
1	48	F	<0.5	NA	?
2	75	F	<0.5	0	No
3	57	M	5	0	No
4	68	M	9	NA	?
5	70	F	<0.5	3c	Yes
6	65	F	<0.5	NA	?
7	35	M	<0.5	3b	Yes
8	70	M	<0.5	3b	Yes
9	75	M	3	1	No

F, female; M, male; NA, not applicable.
[1]All DH patients had IgA deposits in perilesional tissue.

APPENDIX B

Table 2. Evaluated CS patients

CS Patient no.	Sex	Age	Disease duration (years)	Marsh score	Villous atrophy
1	M	13	0.5	3c	Yes
2	F	71	0	3b	Yes
3	F	64	0	3c	Yes
4	M	58	0.5	3b	Yes
5	F	48	0	3c	Yes
6	F	32	0	3c	Yes
7	F	64	0	3c	Yes
8	F	37	0	3c	Yes
9	F	40	0	3c	Yes
10	F	58	0.5	3b	Yes
11	M	65	0.25	3b	Yes
12	M	39	0	3a	Yes
13	F	50	0	3a	Yes
14	F	61	0	3c	Yes
15	M	52	0.25	3a	Yes

F, female; M, male.

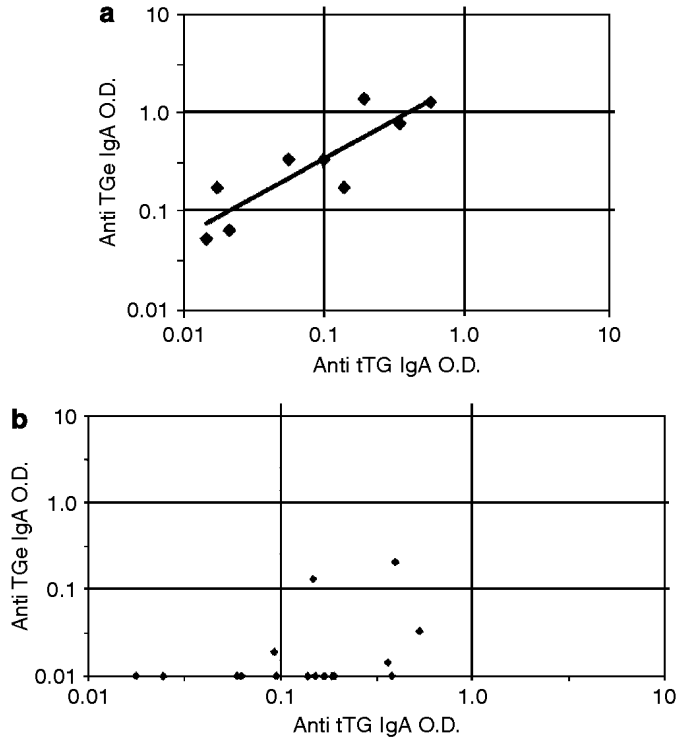

Figure 1. Correlation between anti-tTG IgA and anti-TGe IgA levels in the sera of DH and CS patients on a normal gluten containing diet. OD values for anti-TGe IgA were plotted along the *y* axis and corresponding anti-tTG IgA values for each patient plotted along the *x* axis. All patients were on a normal gluten-containing diet. Nine DH patients were evaluated (**a**) (Pearson's coefficient $r=0.74$), as well as 15 CS patients (**b**) ($r=0.286$).

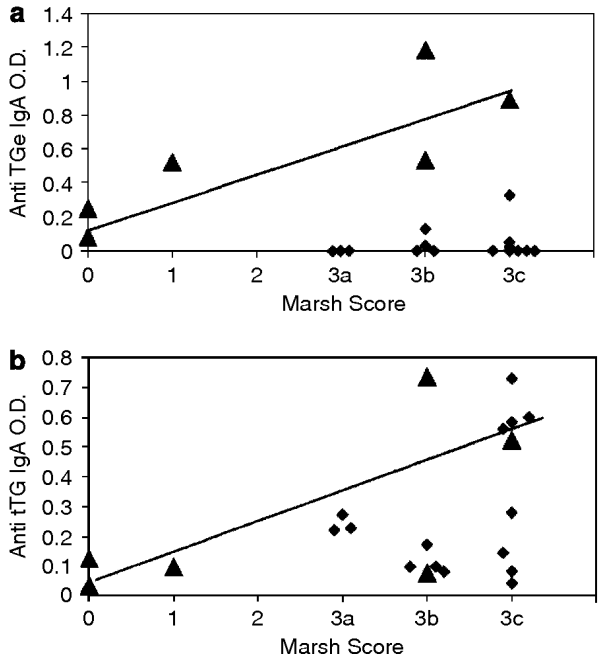

Figure 2. Comparing Marsh Scores with the levels of anti-tTG IgA and anti-TGe IgA in DH and CS patients. Marsh scores for each DH (▲) and each CS patient (◆) were determined and plotted on the *x* axis. OD values for the corresponding anti-TGe IgA level (**a**) and for the corresponding anti-tTG IgA level (**b**) for each DH and each CS patient was plotted on the *y* axis. All patients were on a normal gluten-containing diet. Trend lines are provided for the DH patients (solid). For anti-TGe IgA in DH, $r=0.82$, for anti-tTG IgA in DH, $r=0.7$. For anti-TGe IgA in CS, $r=0.21$, for anti-tTG IgA in CS, $r=0.35$.

concentration of both antibodies in CS patients is independent of the degree of villous atrophy.

It is of great interest then, to understand how and why these antibodies are generated in both DH patients and celiac patients. Clearly, the consumption of gluten triggers symptoms in both diseases, but the mechanisms behind the production of these specific autoantibodies still remains enigmatic. It is possible that the catalyst(s) for the production of anti-tTG IgA and anti-TGe IgA in DH is (are) located in the intestine and is (are) associated with intestinal damage. Future work should be devoted to better understanding what these catalysts (beyond the ingestion of wheat) are. It would also be crucial to determine if the production of these transglutaminase-specific antibodies is necessary for the development of autoimmune pathology in both the small intestine and skin, or if they are they solely consequences of pathology in the intestine.

MATERIALS AND METHODS

Case definition

DH was diagnosed based upon the presence of pruritic papulovesicular lesions and the presence of granular IgA deposits in perilesional skin. Celiac sprue was defined as significant villous atrophy.

At least four endoscopic biopsies from distal duodenum were formalin fixed and paraffin embedded. Sections (5 μm) were stained with hematoxylin and eosin and scored based on the Marsh system.

Detection of anti-TGe IgA and anti-tTg IgA

Anti human tTG ELISA kits (The Binding Site Inc., San Diego, CA) and anti TGe ELISA kits (Alpco, Windham, NH) were used to measure the level of anti-tTG IgA and anti-TGe IgA in the sera of patients. All OD values were normalized by subtracting the value of the negative control provided in the kit. This would mean that any sample that had an OD value greater than the negative control was positive for the antibody tested.

Statistical analysis

Pearson's correlation coefficients were calculated using Microsoft Excel. This study was conducted according to the Declaration of Helsinki Principles and approved by the Mayo Foundation Institutional Review Board. Also, patients had provided consent before the study.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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